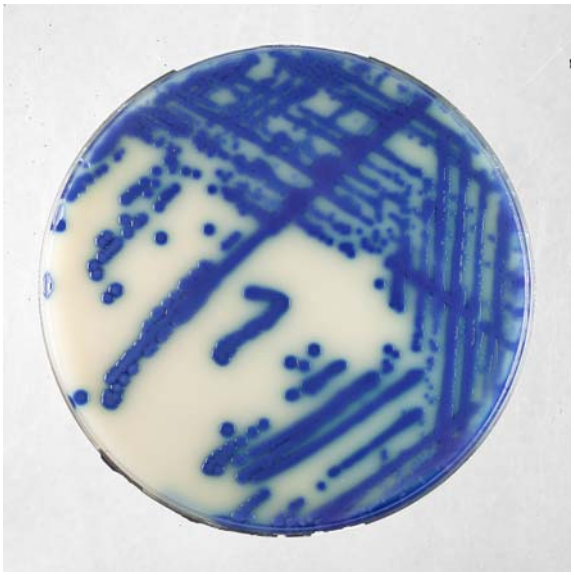
ChromArt

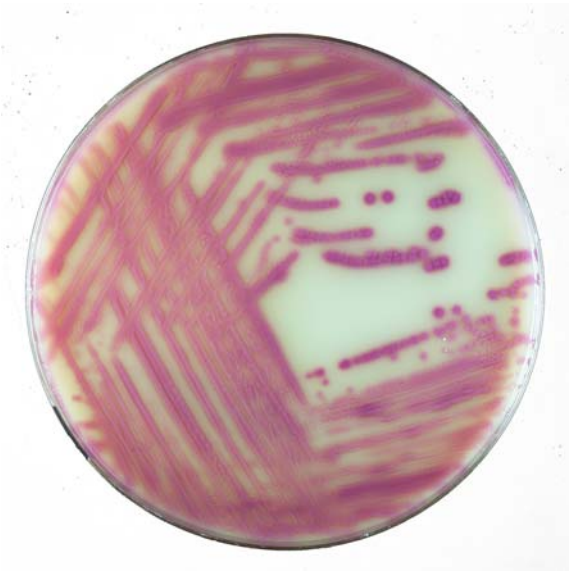
CRE – ESBL MEDIA

CRE-ESBL BASE - CRE SUPPLEMENT - ESBL SUPPLEMENT

Cultural response



Carbapenems resistant *Klebsiella pneumoniae*



ESBL producing *Escherichia coli*

Intended use

CRE-ESBL Base: medium base to be supplemented with CRE Supplement for the detection of carbapenems resistant Gram-negative bacteria or with ESBL Supplement for the detection of ESBL producing *Enterobacteriaceae* in clinical specimens.

CRE Supplement: freeze-dried selective supplement to be used with CRE-ESBL Base for the detection of carbapenems resistant Gram-negative bacteria in clinical specimens.

ESBL Supplement: freeze-dried selective supplement to be used with CRE-ESBL Base for the detection of ESBL producing *Enterobacteriaceae* in clinical specimens.

CRE and ESBL ready to use media: ready to use plates for the detection of carbapenem-resistant Gram-negative bacteria in clinical specimens (ChromArt CRE) or for the detection of ESBL producing *Enterobacteriaceae* in clinical specimens (ChromArt ESBL).

Principle of the method and explanation

The emergence of multi-resistant Gram-negative bacilli (MRGN) creates a challenge in the treatment of nosocomial infections. The prevention and epidemiological monitoring of infections involve the simultaneous application of a number of strategies including the detection of carriers. CRE and ESBL Media are useful tools for the active surveillance of MRGN infections.

CRE-ESBL Base is an improved diagnostic medium useful for the isolation and direct presumptive identification of some Gram negative pathogens: *E.coli, Enterobacter-Klebsiella-Serratia-Citrobacter* (KESC) , *Proteus-Morganella-Providencia.*

The differentiation between the different bacterial species or genus is achieved by:

- a chromogenic substrate for β-galactosidase which is split with the liberation of an insoluble pink dye.
- a chromogenic glucopyranoside derivative which is split by β-glucosidase with the formation of an insoluble blue dye.
- Tryptophan for the detection of tryptophan deaminase of Proteus spp. Morganella spp., Providencia spp.

CRE Supplement contains a carbapenem and a mixture of antimicrobials which enable the selective isolation of carbapenems resistant Gram negative bacteria.

ESBL Supplement contains a third-generation cephalosporin

Typical formulas

Dehydrated medium
(PER LITRE OF PURIFIED WATER)*
REF 408025 **CRE-ESBL BASE**

Peptones	16.00
Growth factors	5.00
Opacifier substance	10.00
Tryptophan	2.00
Chromogenic mix	0.40
Agar	16.00

* the medium can be adjusted and/or supplemented to meet performances criteria

Selective Supplement
(vial content for 500 ml of medium base)
REF 4240082 **CRE SUPPLEMENT**
Antimicrobials mix.....0.21 g/vial

Selective Supplement
(vial content for 500 ml of medium base)
REF 4240080 **ESBL SUPPLEMENT**
Antimicrobials mix.....0.21 g/vial

ChromArt CRE: ready to use plates
(PER LITRE OF PURIFIED WATER)*
REF 54 8015

Peptones	16.00
Growth factors	5.00
Opacifier substance	10.00
Tryptophan	2.00
Chromogenic mix	0.40
Agar	16.00
Antimicrobials mix	0.42

* the medium can be adjusted and/or supplemented to meet performances criteria

ChromArt ESBL : ready to use plates
(PER LITRE OF PURIFIED WATER)*
REF 54 8020

Peptones	16.00
Growth factors	5.00
Opacifier substance	10.00
Tryptophan	2.00
Chromogenic mix	0.40
Agar	16.00
Antimicrobials mix	0.42

* the medium can be adjusted and/or supplemented to meet performances criteria

Directions for preparation of CRE-ESBL Base

Suspend 49.4 g of CRE-ESBL Base (REF 408025) in 1000 ml of cold purified water. Heat to boiling to dissolve completely. Sterilize by autoclaving at 121°C for 15 minutes and cool to 45-50°C.

Directions for preparation of CRE Medium

Dissolve the contents of one vial of CRE Supplement (REF 4240082) with 5 ml of sterile purified water. Add to 500 ml of CRE-ESBL Base (REF 408025), autoclaved and cooled to 45-50°C. Mix well and distribute into sterile Petri dishes.

Directions for preparation of ESBL Medium

Dissolve the contents of one vial of ESBL Supplement (REF 4240080) with 5 ml of sterile purified water. Add to 500 ml of CRE-ESBL Base (REF 408025), autoclaved and cooled to 45-50°C. Mix well and distribute into sterile Petri dishes.

Physical characteristics

Dehydrated medium appearance: grey, fine, homogeneous, free-flowing powder.
Prepared medium appearance: pale grey, opaque
Reconstituted CRE supplement appearance: light red opalescent.
Reconstituted ESBL supplement appearance: light red opalescent.
Final pH of CRE complete medium: 7.2 ± 0.2
Final pH of ESBL complete medium: 7.2 ± 0.2

Specimens

CRE Medium and ESBL medium can be used for the inoculation of feces or rectal swabs or other clinical specimens or cultures obtained from clinical specimens. The specimens should be sent to the laboratory as soon as possible and managed according to the good laboratory practices.

Technique

Inoculate the specimen or the culture of the specimen directly on the surface of the plate of CRE Medium and/or ESBL Medium.
Spread the inoculum on the surface of the culture medium plate
Incubate at 35-37°C for 18-24 hours. Negative plates should be re-incubated for an additional 24 hours.
After incubation observe for the growth and the appearance of colonies and perform the presumptive identification with the following scheme.

Colonies	**Presumptive identification on CRE Medium**	**Presumptive identification on ESBL Medium**
Large pink colonies	Carbapemens resistant *E.coli*	ESBL producing *E.coli*
Blue, blue-green, purple-blue, grey-purple colonies	Carbapemens resistant *Klebsiella, Enterobacter, Serratia, Citrobacter*	ESBL producing *Klebsiella, Enterobacter, Serratia, Citrobacter*
Brown colonies with brown halo	Carbapemens resistant *Proteus-Morganella-Providencia*	ESBL producing *Proteus-Morganella-Providencia*
White colonies	Carbapenems resistant *Acinetobacter* or other Gram negative non fermenting bacteria	ESBL producing *Acinetobacter, Pseudomonas* or other Gram negative non fermenting bacteria

User's Quality Control

The user is responsible for performing the quality control according to national regulations.
The following strains are given as an example for the user's quality control:
CRE medium
Productivity control: *K.pneumoniae* ATCC BAA-1705: good growth, blue colonies.
Selectivity control: *E.coli* ATCC 25922, *C.albicans* ATCC 10231: inhibited.
ESBL Medium
Productivity control *K.pneumoniae* SHV-18 ATCC 700603: good growth, blue colonies.
Selectivity control *: E.coli* ATCC 25922, *C.albicans* ATCC 10231: inhibited.

Limitations

- The presumptive identification obtained with CRE Medium and ESBL Medium should be confirmed with appropriate diagnostic tests: molecular biology, discs/tablets, biochemistry etc.
- Carbapenemase negative strains with membrane impermeability due to the porines loss , may growth on CRE Medium.
- Strains overproducing chromosomal AmpC beta-lactamase may grow on ESBL Medium.
- OXA-48 carbapenemase producing strains may fail to grow on ESBL Medium.
- The results obtained with CRE Medium and ESBL Medium should be considered in conjunction with the patient's history, the specimen source, the colonies morphology, the microscopic observations and the results of the confirmatory tests.

Performances evaluation of CRE Medium

CRE medium was evaluated at a North Italy Hospital using characterized strains of clinical origin. CRE Medium plates and Tryptic Soy Agar plates were directly inoculated with calibrated bacterial suspensions and incubated at 37°C for 18-24 hours.
The following strains of clinical origin have been used for the performances evaluation of CRE Medium: 110 strains of carbapenems resistant Gram-negative bacteria, 50 strains of 3th generation cephalosporin resistant Enterobacteria.
The results are summarized in the tables.

Table 1: results obtained with 97 carbapenemase producing strains (Ambler Class A, B, D)

Resistance mechanism	N° of strains	Growth on CRE Medium	Growth on Tryptic Soy Agar
KPC strains	60	60	60
KPC+ESBL strain	1	1	1
OXA strains	12	12	12
VIM strains	15	15	15
NDM strains	3	3	3
IMP strains	4	4	4
MBL strains	2	2	2
Total	**97**	**97**	**97**

Table 2: Results obtained with 13 carbapenems resistant Gram negative strains (membrane impermeability).

Resistance mechanism	N° of strains	Growth on CRE Medium	Growth on Tryptic Soy Agar
AmpC + porines loss	5	3	5
ESBL + porines loss	8	8	8
Total	**13**	**11**	**13**

Table 3: Results obtained with 50 carbapemens susceptible strains .

Resistance mechanism	N° of strains	Growth on CRE Medium	Growth on Tryptic Soy Agar
AmpC strains	10	0	10
ESBL	40	0	40
Total	**50**	**0**	**50**

Table 4 : sensitivity and specificity referred to two different targets

	Target: carbapenemase production	Growth on CRE Medium	Target: resistance to carbapenems	Growth on CRE Medium
True positive strains	97	97	110	108
True negative strains	63	11	50	0
False positive strains		11		0
False negative strains		0		2

Sensitivity	100%		98.2%
Specificity	85.1%		100%

The data demonstrate that CRE Medium is able to detect carbapenems resistant Gram negative bacteria with high sensitivity (98,2%) and specificity of 100% because it inhibits the growth of carpapenems susceptible beta-lactamase producing microorganism with different resistance mechanisms (e.g. ESBL and AmpC overproducers). If the relevant target of CRE medium is the detection of carbapenamase production, the specificity is 85,1% because the medium allows the growth of carbapenems resistant bacteria due to membrane impermeability (loss of porines).

Performances evaluation of ESBL Medium

ESBL medium was evaluated at a North Italy Hospital using human clinical specimens (urines, blood, cephalorachidian fluid). ESBL Medium plates were directly inoculated with the specimens and the results reading was performed after 18-24 hours incubation at 37°C.
The results are summarized in the tables.

Table 1: Results obtained with 2538 clinical specimens

	N°	Isolated Enterobacteria strains	Strains confirmed as ESBL producers*	Strains confirmed as ESBL non-producers*
Urines	2500	736	79	657
Other specimens°	38	37	6	31
Total	2538	773	85	688
Growth on ESBL Medium			**84**	**12****

° 37 blood cultures, 1 cephalorachidian liquid
* ESBL production has been detected with the double disk technique
**: 9 strains have been identified as AmpC overproducers

Sensitivity: 98.82%
Specificity: 98.29%

The data demonstrate that ESBL Medium is able to detect ESBL producing *Enterobacteriaceae* with high sensitivity and specificity.

Warning and precautions

- The culture medium and supplements here described are not classified as dangerous according to European and National Directives and laws and do not contain dangerous ingredients at concentration ≥1%.
- The culture medium here described contains products from animal origin (peptones). Download from the web site www.biolifeitaliana.it the document for Risk Assessment of Products from Animal Origin.
- CRE-ESBL Base must be used only with CRE Supplement or ESBL Supplement. CRE Supplement or ESBL Supplement must be used only for the supplementation of CRE-ESBL Base.
- The culture medium and supplements here described are for *in vitro* diagnostic use only and should be used by trained Laboratory technicians.
- All the clinical specimens should be considered infectious: observe approved biohazard precautions and aseptic techniques.
- Allow the plates to reach room temperature before use.
- Sterilize all biohazard waste before disposal.
- Download the Quality Control Certificate from the website www.biolifeitaliana.it

Storage

Chrom*Art* CRE-ESBL Medium Base : upon receipt store at 2°C to 8°C and protect from the direct light. The expiry date applies to the products in the intact packaging when stored as directed.
Chrom*Art* CRE Supplement, Chrom*Art* ESBL Supplement : upon receipt store at 2°C to 8°C The expiry date applies to the un-opened vials.

References

1- Evaluation of ChromArt CRE (Biolife) for the detection of carbapenems resistant Gram-negative bacteria. Silvia Bracco, Carola Mauri, Elisa Meroni, Luigi Principe, Beatrice Pini, Francesco Luzzaro. XLIII AMCLI Congress, 2014.
2- Evaluation of ChromArt ESBL (Biolife) for the detection of ESBL producing *Enterobacteriaceae* in clinical specimens. Cristina Comi, Silvia Bracco, Laura Colombo, Patrizia Bartesaghi, Rita Barletta, Martha Silva, Francesco Luzzaro. XLIII AMCLI Congress, 2014.

Ordering information

Product	Type	Cat. N°	Pack size
Chrom*Art* CRE – ESBL BASE	DCM	4080252	500 g (10.1 L)
Chrom*Art* CRE – ESBL BASE	DCM	4080254	5000 g (101 L)
Chrom*Art* CRE SUPPLEMENT	Supplement for 500 ml of medium	4240082	10 x 5 ml
Chrom*Art* ESBL SUPPLEMENT	Supplement for 500 ml of medium	4240080	10 x 5 ml
Chrom*Art* CRE	Ready to use plates	548015	20 plates
ChromArt ESBL	Ready to use plates	548020	20 plates

IVD CE

Biolife Italiana S.r.l. Viale Monza 272, 20128 Milano.